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Perturbed T Cell IL-7 Receptor Signaling in Chronic Chagas Disease

M. Cecilia Albareda,*,† Damián Perez-Mazliah,* M. Ailén Natale,* Melisa Castro-Eiro,* María G. Alvarez,† Rodolfo Viotti,† Graciela Bertocchi,† Bruno Lococo,† Rick L. Tarleton,‡ and Susana A. Laucella*,†

We have previously demonstrated that immune responses in subjects with chronic *Trypanosoma cruzi* infection display features common to other persistent infections with signs of T cell exhaustion. Alterations in cytokine receptor signal transduction have emerged as one of the cell-intrinsic mechanisms of T cell exhaustion. In this study, we performed an analysis of the expression of IL-7R components (CD127 and CD132) on $CD4^+$ and $CD8^+$ T cells and evaluated IL-7–dependent signaling events in patients at different clinical stages of chronic chagasic heart disease. Subjects with no signs of cardiac disease showed a decrease in $CD127^+$ $CD132^+$ cells and a reciprocal gain of $CD127^-CD132^+$ in $CD8^+$ and $CD4^+$ T cells compared with either patients exhibiting heart enlargement or uninfected controls. *T. cruzi* infection, in vitro, was able to stimulate the downregulation of CD127 and the upregulation of CD132 on T cells. IL-7–induced phosphorylation of STAT5 as well as Bcl-2 and CD25 expression were lower in *T. cruzi*–infected subjects compared with uninfected controls. The serum levels of IL-7 were also increased in chronic chagasic patients. The present study highlights perturbed IL-7/IL-7R T cell signaling through STAT5 as a potential mechanism of T cell exhaustion in chronic *T. cruzi* infection. *The Journal of Immunology*, **2015, 194: 000–000.**

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Chagas disease, caused by *Trypanosoma cruzi* infection, represents a major public health problem in Latin America (1). As a consequence of migration flows, the disease is also found in nonendemic countries, becoming a global health problem (2). T cells play a major role in the immune control of *T. cruzi*. We have shown that *T. cruzi*–infected subjects in the chronic phase of the infection have increased frequencies of fully differentiated and preapoptotic memory T cells along with decreased levels of naive T cells in the peripheral circulation, reflecting a constant activation of the immune system in association with this decades-long infection (3–5). These immune dysfunctions of the total T cell compartment were associated with decreased frequencies of IFN-γ– and IL-2–producing T cells in response to *T. cruzi* Ags in patients with severe myocardiopathy (3, 6, 7).

IL-7 plays an important role in the maintenance of naive and memory T cell compartments, positively regulating the survival, differentiation, and proliferation of T cells. The IL-7 cell surface receptor is composed of the specific IL-7Rα (CD127) chain and the common γ-chain (CD132 or γ_c), which is shared with other cytokines of the γ_c family (8). The regulation of the two IL-7R chains is different; that is, whereas CD127 is downregulated upon T cell activation, the CD132 chain is rapidly upregulated (9). In persistent infections, reduced expression of the IL-7R and an inability to effectively respond to this cytokine has been associated with defective Ag-independent homeostatic self-renewal and loss of pathogen-specific T cells (10–15).

In view of the key role of IL-7 in controlling T cell homeostasis, alterations in IL-7 signaling might also be involved in the impaired T cell responses observed in patients with chronic Chagas disease, as supported by the lower expression of CD127 on $CD4^+$ T cells in chronically *T. cruzi*–infected subjects with severe cardiomyopathy (4). In the present study, we performed an analysis of the expression of IL-7R components and evaluated IL-7–dependent signaling events in patients in different clinical stages of chronic Chagas heart disease. The findings reported support the notion that *T. cruzi* infection perturbed the IL-7/IL-7R T cell signaling through STAT5 and thus represents another potential mechanism of T cell exhaustion in chronic *T. cruzi* infection.

Materials and Methods

Selection of study population

Subjects were recruited at the Chagas Disease Section, Cardiology Department, Hospital Interzonal General de Agudos Eva Perón (Buenos Aires, Argentina). *T. cruzi* infection was determined by a combination of indirect immunofluorescence assay, hemagglutination, and ELISA tests performed at the Diagnosis Department of Instituto Nacional de Parasitología Dr. M. Fatala Chaben. Chronic Chagas disease subjects were evaluated clinically and grouped according to a modified version of the Kuschnir grading system (16). Group 0 (G0, $n = 13$; mean age ± SD, 50 ± 6 y) included seropositive individuals exhibiting a normal electrocardiogram and normal echocardiograph, group 1 (G1, $n = 12$; mean age ± SD, 49 ± 14y) included seropositive individuals with a normal echocardiograph but abnormalities in the electrocardiogram, and groups 2 and 3 (G2 and G3, $n = 12$; mean age ± SD, 56 ± 11 y) included seropositive individuals with electrocardiogram abnormalities and heart enlargement (G2) and clinical or radiological evidence of heart failure (G3). All patients were untreated with respect to *T. cruzi* infection, with the exception being one patient in the G2 patient group who received treatment with benznidazole 10 y prior to this study and who remained seropositive for *T. cruzi* infection. The

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Abbreviations used in this article: γ_c, common γ-chain; G1–3, groups 1–3; MFI, mean fluorescence intensity.

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uninfected control group ($n = 10$; mean age ± SD, 47 ± 10 y) consisted of aged-matched healthy white natives from Argentina who have always resided in areas nonendemic for *T. cruzi* infection and who have negative serology for *T. cruzi* infection. *T. cruzi*–infected subjects and uninfected controls with hypertension, ischemic heart disease, cancer, HIV infection, syphilis, diabetes, arthritis, or serious allergies were excluded from this study. This study was approved by the Institutional Review Boards of the Hospital Interzonal General de Agudos Eva Perón and the University of Georgia (Athens, GA). Signed informed consent was obtained from all individuals prior to inclusion in the study.

Collection of PBMCs and sera

Approximately 50 ml blood was drawn by venipuncture into heparinized tubes (Vacutainer; Becton Dickinson, San Jose, CA). PBMCs were isolated by density gradient centrifugation on Ficoll-Hypaque (Amersham Biosciences, Umea, Sweden) and resuspended in RPMI 1640 (Mediatech, Herndon, VA) supplemented with 10% heat-inactivated FCS (HyClone Laboratories, Logan, UT). Blood to be used for serum component analysis was allowed to coagulate at 37°C and centrifuged at $1000 \times g$ for 15 min. Non-hemolyzed serum was separated and aliquots were stored at −70°C until use.

mAbs

PE-conjugated anti–Bcl-2 (clone Ms IgG1), fluorescein (FITC)-conjugated anti-CD25 (clone M-A251), PE-conjugated anti-CD132 (clone AG184), allophycocyanin (clone RPA-T4)- and PerCP (clone RPA-T4)-conjugated anti-CD4, PerCP (clone SK1)- or FITC (clone HIT8a)-conjugated anti-CD8, Alexa Fluor 647–conjugated (clone HIL-7R-M21) anti-CD127 or PE (clone HIL-7R-M21), as well as PE-conjugated anti–p-STAT5 (clone pY694) were purchased from BD Pharmingen (San Diego, CA).

Expression of CD127 and CD132 in PBMCs from chronically T. cruzi*–infected subjects*

One million PBMCs were stained with anti-CD4 PerCP, anti-CD8 FITC, anti-CD127 Alexa Fluor 647, and anti-CD132 PE for 30 min on ice. Thereafter, the cells were washed and resuspended in PBS containing 2% paraformaldehyde. Data were acquired on a FACSCalibur flow cytometer (BD Biosciences) and analyzed with FlowJo version 6.3 software (Tree Star, San Carlos, CA).

Expression of IL-7R components on the T. cruzi*–specific T cells in vitro*

PBMCs (2×10^6) were cocultured with Vero cell culture–derived trypomastigotes (Brazil strain) at a ratio of 1:1 cells/parasites in a 24-well plate at 37°C in 5% CO_2 atmosphere during 48 h. A ratio of 1:5 cells/parasites was also tested to set up the appropriate conditions for in vitro infection with *T. cruzi*. The supernatants were removed, washed with staining buffer, and labeled with anti-CD4 PerCP, anti-CD8 FITC, anti-CD127 Alexa Fluor 647, and anti-CD132 PE for 30 min on ice. Cells were then washed and resuspended in PBS containing 2% paraformaldehyde. Data were acquired on a FACSCalibur flow cytometer (BD Biosciences) and analyzed with FlowJo version 6.3 software (Tree Star).

Intracellular p-STAT5 assay

PBMCs (2×10^6) were cultured overnight in serum-free medium (AIM-V; Invitrogen, Carlsbad, CA) followed by a 15-min incubation with 100 ng/ml recombinant human IL-7 (BD Pharmingen) at 37°C, 5% CO_2. Thereafter, cells were washed with staining buffer (PBS, 4% FBS) labeled with CD4 and CD8 mAbs for 20 min on ice and immediately fixed by adding an equal volume of prewarmed 4% paraformaldehyde for 10 min at 37°C. After centrifugation and paraformaldehyde removal by aspiration, cells were permeabilized by adding 1 ml 90% ice-cold methanol for 30 min on ice and washed twice with staining buffer. Lastly, the cells were incubated at room temperature for 30 min with anti–p-STAT5 (20 μl per test according to the instructions of the manufacturer). Data were acquired on a FACSCalibur flow cytometer (BD Biosciences) and further analyzed with FlowJo version 6.3 software (Tree Star). IL-7–induced STAT5 phosphorylation (change in percentage of p-STAT5$^+$) for CD4$^+$ and CD8$^+$ T cells was measured by the difference in the percentage of p-STAT5$^+$ cells between IL-7–stimulated and unstimulated samples.

Measurement of serum IL-7 cytokine

Serum levels of IL-7 were measured, in duplicate, using an IL-7 human SimpleStep ELISA kit (Abcam) according to the instructions of the manufacturer.

Evaluation of IL-7 functional responses

PBMCs (2×10^6) were cultured in complete RPMI medium in the presence or absence of 10 ng/ml recombinant human IL-7 for 2 d. After the incubation, cells were labeled with anti-CD4 (allophycocyanin), anti-CD8 (PerCP), and anti-CD25 (FITC) for 30 min on ice followed by permeabilization with Cytofix/Cytoperm solution (BD Pharmingen) according to the manufacturer's instructions. Thereafter, cells were labeled with anti–Bcl-2 (PE) for 30 min at 4°C, washed twice with Perm/Wash solution, and resuspended in PBS containing 2% paraformaldehyde. Data were acquired on a FACSCalibur flow cytometer (BD Biosciences) and further analyzed with FlowJo version 6.3 software (Tree Star). Lymphocytes were gated in side scatter versus forward scatter light parameters. Induction of Bcl-2 and CD25 expression was measured by subtracting the mean fluorescence intensity (MFI) or percentages of Bcl-2– and CD25-expressing T cells in unstimulated cultures from those in IL-7–stimulated cultures.

Statistical analysis

Differences among groups were evaluated by ANOVA followed by a Bonferroni test for multiple comparisons or by a test for lineal trend. A Mann–Whitney U test was used to evaluate the differences between *T. cruzi*–infected and uninfected subjects. A paired t test was used to compare the expression of the IL-7R components prior to and after in vitro *T. cruzi* infection. Correlation between variables was explored with the Spearman test. Differences were considered statistically significant when $p \leq 0.05$.

Results

Expression of IL-7R components in subjects with chronic T. cruzi *infections*

The effect of *T. cruzi* infection on the expression of the IL-7R components, CD127 and CD132, was evaluated in CD8$^+$ and CD4$^+$ T cells from subjects with *T. cruzi* infections of >20 y in length. Two main subpopulations (CD127$^+$CD132$^+$ and CD127$^-$CD132$^+$) of CD8$^+$ and CD4$^+$ T cells out of the four possible subsets based on the expression of CD127 and CD132 IL-7R components were observed in chronically *T. cruzi*–infected subjects. In subjects in the G0 group the CD8$^+$ T cell subset showed a decrease in CD127$^+$CD132$^+$ cells and a reciprocal gain of CD127$^-$CD132$^+$ cells in comparison with patients with heart enlargement (G2 and G3 clinical groups) (Fig. 1A, 1B, 1E). Of note, no significant differences in CD8$^+$CD127$^+$CD132$^+$ and CD8$^+$CD127$^-$CD132$^+$ T cell populations were found between patients with severe cardiac dysfunction and uninfected controls (Fig. 1A, 1B, 1E). A similar trend was observed in the CD4$^+$ T cell compartment, with diminished CD127$^+$CD132$^+$CD4$^+$ T cells along with increased CD127$^-$CD132$^+$CD4$^+$ T cells in the G0 patient group (Fig. 1C–E). One consequence of T cell activation is the downregulation of CD127. Thus, these findings suggest a differential response to T cell activation in G0 subjects relative to patients with severe disease.

Modulation of IL-7R components in T cells after T. cruzi *infection in vitro*

To assess whether *T. cruzi* infection might modulate the IL-7R components, the expression of CD127$^+$ and CD132$^+$ was measured on T cells after in vitro infection of PBMCs with *T. cruzi*. A downregulation of CD127 and a reciprocal gain of CD132 in CD4$^+$ and CD8$^+$ T cells was observed in all the subjects evaluated, supporting the activation of the IL-7R (Fig. 2) after infection with *T. cruzi*.

Decreased IL-7–induced STAT5 phosphorylation in T cells from chronically T. cruzi*–infected subjects*

A common signaling event that occurs rapidly after ligation of γ_c cytokines to their receptors is the JAK-dependent phosphorylation of the STAT5 (17). To evaluate potential defects in IL-7R–mediated signaling, IL-7–induced phosphorylation of STAT5 was measured in CD4$^+$ and CD8$^+$ T cells from *T. cruzi*–infected subjects with different degrees of disease severity and in uninfected controls.

The basal number of CD4$^+$ and CD8$^+$ T cells expressing p-STAT5$^+$ in G2 and G3 patients was higher compared with G0, G1, and

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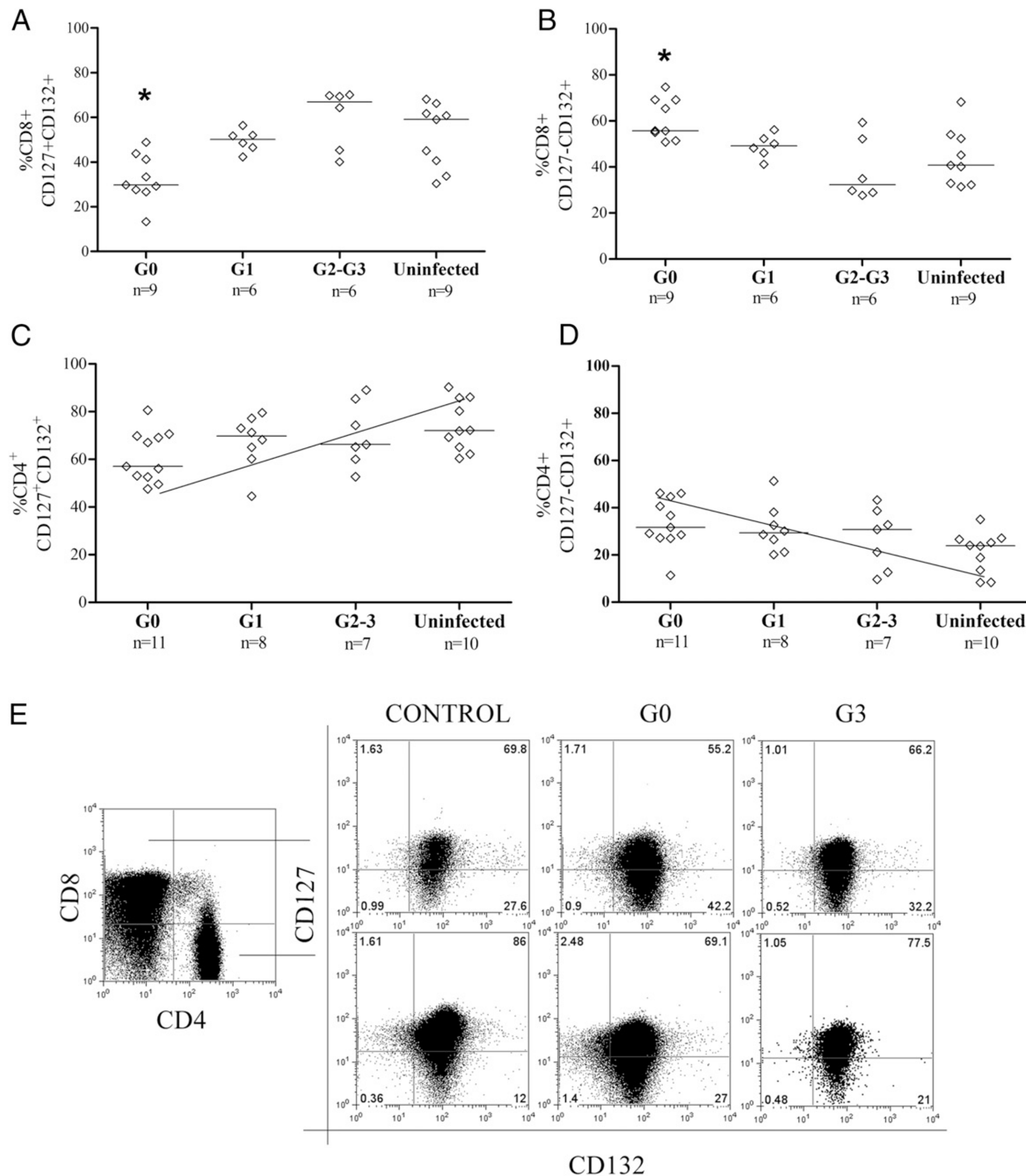

FIGURE 1. Cell surface expression of IL-7R components in subjects with chronic *T. cruzi* infection. PBMCs were stained for CD8, CD4, CD127, and CD132 and analyzed by flow cytometry. Lymphocytes were gated by side scatter versus forward scatter light. Each point represents the expression of CD127 and CD132 on the total $CD8^+$ and $CD4^+$ T cell compartments in one patient. Median values are indicated by the horizontal lines. Differences among groups were evaluated by ANOVA followed by a Bonferroni test. Statistically significantly differences (*$p < 0.05$) in the frequencies of $CD8^+CD127^+CD132^+$ (**A**) and $CD8^+CD127^-CD132^+$ (**B**) in G0 patients versus uninfected or G2-G3 clinical groups. (**C** and **D**) Positive and negative trend in the percentages of $CD127^+$ $CD132^+$ and $CD127^-CD132^+$ $CD4^+$ T cells, respectively, as clinical stage becomes more severe. (**E**) Representative dot plots of the expression of CD127 and CD132 in $CD4^+$ and $CD8^+$ T cells from an uninfected control, an asymptomatic patient (G0), and a severe cardiomyopathy patient (G3). The numbers in each quadrant represent the percentage of expression of each of the four possible subsets based on the expression of CD127 and CD132 IL-7R components.

uninfected controls (Fig. 3A, 3B, 3E). The percentages of $CD4^+$p-$STAT5^+$ and $CD8^+$p-$STAT5^+$ T cells after IL-7 stimulation were significantly lower in all groups of *T. cruzi*–infected subjects compared with uninfected controls (Fig. 3C–E). Of note, we observed a trend toward lower responsiveness to IL-7 in association with the increased severity of disease (CD4 $p = 0.0005$ and CD8 $p = 0.0001$, respectively; Fig. 3C, 3D). In contrast, the analysis of basal or IL-7–induced MFI of p-STAT5 did not show any differences between *T. cruzi*–infected subjects and uninfected controls (Supplemental Table I).

The expression of the IL-7R components (CD127 and CD132) on $CD4^+$ or $CD8^+$ T cells was not correlated with the levels of p-$STAT5^+$ T cells following IL-7 stimulation (data not shown). In contrast, higher basal phosphorylation levels were correlated with lower magnitudes of IL-7–induced STAT5 phosphorylation ($CD4^+$ T cells, $r = -0.6$, $p < 0.0001$ and $CD8^+$ T cells, $r = -0.75$, $p < 0.0001$; data not shown). Altogether, these findings indicate altered IL-7/IL-7R–mediated signaling in chronic *T. cruzi* infection, increasing as cardiac disease becomes more severe.

IL-7 serum levels in chronic Chagas disease patients

Taking into account that IL-7 is one of the STAT5-activating cytokines, the levels of circulating IL-7 were measured in 30

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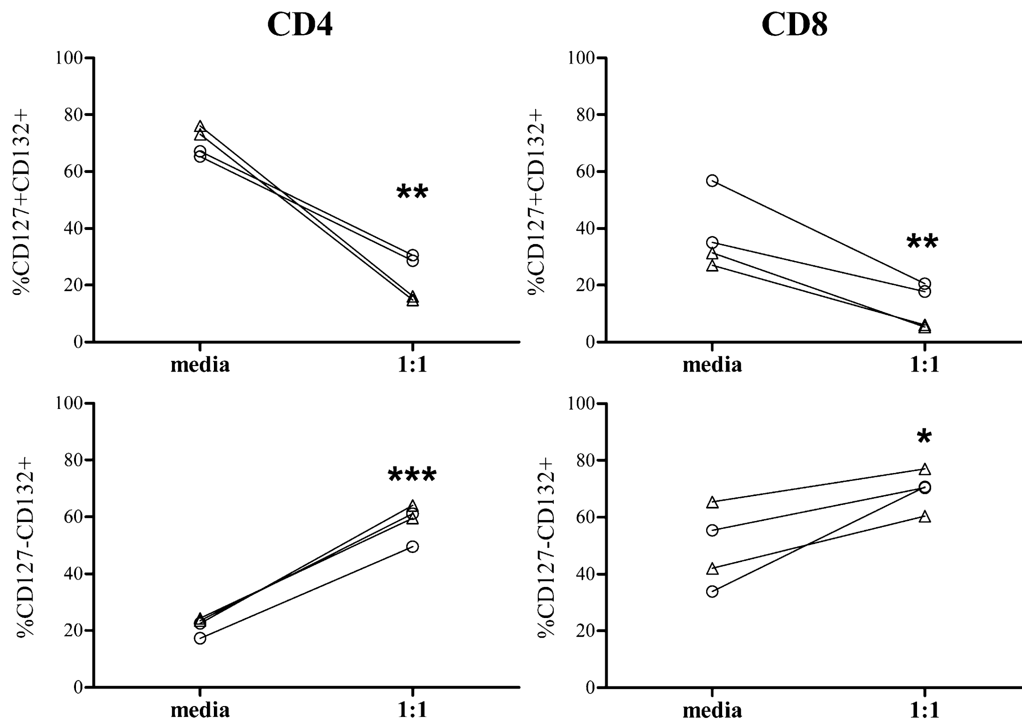

FIGURE 2. Modulation of the IL-7R components after in vitro infection of T cells with *T. cruzi*. PBMCs from two chronic Chagas disease subjects (○) and two uninfected controls (△) were cocultured with *T. cruzi* in a 1:1 ratio for 48 h, stained for CD8, CD4, CD127, and CD132, and analyzed by flow cytometry. Lymphocytes were gated by side scatter versus forward scatter light. Each point represents the expression of CD127 and CD132 on total $CD8^+$ (*right panel*) and $CD4^+$ (*left panel*) T cells from media and *T. cruzi*–stimulated wells in each subject. *$p < 0.05$, ** $p < 0.01$, ***$p < 0.005$.

chronic Chagas disease patients and 9 uninfected controls. An increased level of IL-7 was found in chronically *T. cruzi*–infected subjects compared with uninfected controls (Fig. 4). There was no correlation between the concentration of IL-7 and the basal or IL-7–induced p-STAT5 expression (data not shown).

Impairment of IL-7 functional responses downstream of STAT5 in chronic Chagas disease patients

IL-7 is known to induce the expression of the survival factor Bcl-2 and the IL-2R α-chain (CD25) through STAT5 (18). To evaluate the functional responses of $CD4^+$ and $CD8^+$ T cells to IL-7 downstream STAT5, the induced expression of Bcl-2 and CD25 was measured. Because Bcl-2 is constitutively expressed, the change in MFI was used to evaluate the induction above the basal levels. The induction of Bcl-2 on $CD4^+$ and $CD8^+$ T cells was reduced in G2 and G3 compared with G0 and G1 patient groups and uninfected controls (Fig. 5A, 5B), whereas baseline Bcl-2 expression on T cells remained unchanged in *T. cruzi*–infected subjects (Supplemental Table I).

The percentage of $CD8^+$ T cells expressing CD25 after the IL-7 stimulation was lower in chronically *T. cruzi*–infected subjects, regardless of the clinical status (Fig. 5D). A trend toward higher basal percentages of $CD25^+CD8^+$ T cells (data not shown) as well as higher MFI of $CD25^+CD8^+$ T cells (Supplemental Table I) as disease becomes more severe were found. Conversely, no alterations were observed either in baseline or IL-7–induced expression of CD25 on $CD4^+$ T cells in *T. cruzi*–infected subjects (Fig. 5C, Supplemental Table I). These findings support that T cell function downstream STAT5 is also perturbed in the face of the chronic infection.

Discussion

Different mechanisms of T cell exhaustion have been demonstrated during chronic infections, including overexpression of inhibitory receptors, altered chemotaxis, homing and adhesion molecules, changes in transcription factor expression, as well as metabolic deficiencies (15). We have previously demonstrated that T cells in subjects with chronic *T. cruzi* infection display signs of immune exhaustion, as shown by the presence of monofunctional *T. cruzi*–specific T cells and a high degree of differentiation of the overall T cell compartment (3, 4, 6, 7). Alterations in signal transduction through cytokine receptors, including IL-7, IL-15, and other γ-chain cytokine signals mediated predominantly by the kinases Jak1, Jak3, and STAT5, have emerged as one of the cell-intrinsic mechanisms of T cell exhaustion during chronic infections (15, 19). The present study shows that chronic *T. cruzi* infection leads to a perturbation of the IL-7/IL-7R–mediated T cell signaling through the STAT5 pathway that is independent of the expression of the IL-7R components.

IL-7 is a critical cytokine that regulates the transition from effector to memory T cells, as it is involved in survival and homeostasis of memory T cells (8, 20). Naive T cells express IL-7R, but IL-7R expression drops on activated T cells as they expand and differentiate during infection. At the peak of expansion, most effector T cells are $IL\text{-}7R^{lo}$; however, a smaller subset of $IL\text{-}7R^{hi}$ cells exists that expresses higher levels of Bcl-2 and preferentially survives to generate long-lived memory T cells with self-renewal capacity (21–24).

T cells in patients with no signs of cardiac disease appeared to retain the capability to regulate the expression of the IL-7R components with a relative loss of $CD127^+CD132^+$ T cells along with an increase in $CD127^-CD132^+$ cells as expected in the context of a process of immune activation. It has been shown that $CD127^-$ $CD132^+$ T cells are mainly comprised by short-lived effector T cells (25), concurring with the high frequencies of total effector CD4 and CD8 T cells found in chronically *T. cruzi*–infected subjects (3, 4). Despite that patients in the chronic phase of the infection, including those with more severe forms of the disease, display an activated immune status (3, 4, 26–29), in the present study we show that the

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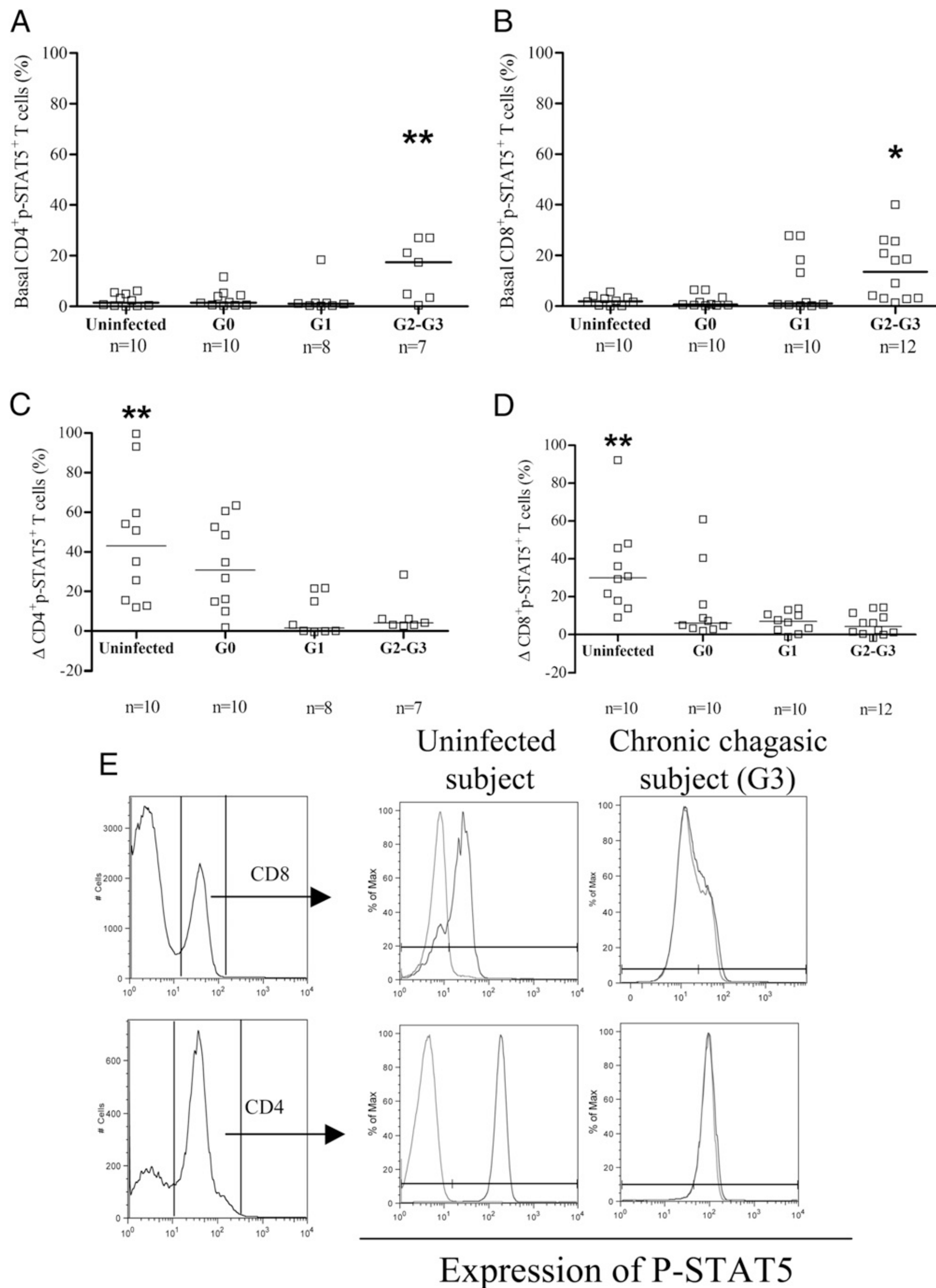

FIGURE 3. STAT5 activation in CD4+ and CD8+ T cells in chronic Chagas disease. PBMCs were stimulated with 100 ng/ml IL-7 and evaluated for p-STAT5+ induction in CD4+ and CD8+ T cells by flow cytometry. Lymphocytes were gated in side scatter versus forward sacatter light. (**A** and **B**) Each point represents the basal levels of CD4+p-STAT5+ and CD8+p-STAT5+ T cells, respectively. Differences among groups were evaluated by ANOVA followed by a Bonferroni test. Median values are indicated by the horizontal lines. (**C** and **D**) Each point represents the induced expression of p-STAT5 for individual subjects calculated as the difference in the percentage of CD4+p-STAT5+ or CD8+p-STAT5+ T cells, respectively, between IL-7–stimulated and unstimulated samples. Differences among groups were evaluated by ANOVA followed by a Bonferroni test. Median values are indicated by the horizontal lines. $*p < 0.05$, $**p < 0.01$. (**E**) Representative CD4+/CD8+ histogram plots of PBMCs from an uninfected control and a *T. cruzi*–infected subject with severe cardiomyopathy. Gray lines indicate the basal expression of p-STAT5 and the black lines the expression of p-STAT5 after IL-7 stimulation.

capability to modulate IL-7R components are impaired in more severe clinical stages. Our experiments of coculture of PBMCs with *T. cruzi* trypomastigotes support that *T. cruzi* can mediate the modulation of the IL-7R components. In our system, IL-7R might have been activated either through the interaction of T cells with APCs displaying *T. cruzi* Ags on their surfaces, or through the action of cytokines released after in vitro infection.

Further evidence of the disturbances in the IL-7/IL-7R pathway is the lower levels of STAT5 phosphorylation in response to IL-7 in association with increased basal STAT5 phosphorylation in patients with cardiomyopathy. Apart from persistent Ag stimulation, upregulated expression of IL-7 and IL-15 might induce a dysregulation in the IL-7/IL-7R pathway (8, 15, 19). Other cytokines or growth factors, such as thymic stromal lymphopoietin, IL-3, IL-5, or IL-9 released as a result of the persistent infection, could also activate STAT5 (30), accounting for the high basal p-STAT5. This upregulation of STAT5 phosphorylation may in turn desensitize the STAT5-dependent signaling pathway, resulting in the lower

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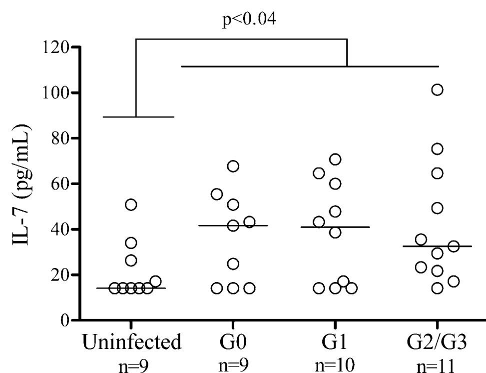

FIGURE 4. Serum IL-7 concentration in chronic Chagas disease patients. Serum levels were measured in 30 chronically *T. cruzi*–infected subjects with different clinical stages of the disease and 9 uninfected controls using an IL-7 capture ELISA assay. Each point represents the IL-7 serum levels in each subject. Median values are indicated by the horizontal lines.

capacity to respond to IL-7 stimulation and to a downregulation of the IL-7R α-chain in these patients.

Although serum levels of IL-7 were increased in chronically *T. cruzi*–infected subjects, we did not observe a correlation between circulating IL-7 and basal or induced p-STAT5 expression. Fonseca et al. (31) have also demonstrated increased mRNA for IL-7 and the CD132 chain of the IL-R in heart tissues from Chagas disease patients with severe cardiomyopathy, supporting a putative role for IL-7 in the alteration of IL-7R components in the chronic phase of *T. cruzi* infection. A role for IL-5 and IL-9 in the perturbation of the IL-7 signaling pathway cannot be ruled out because these cytokines have been also detected in sera from patients with chronic cardiomyopathy (32, 33). In human HIV infection, impaired IL-7/IL-7R signaling was correlated with the levels of basal phosphorylation, viral load, and activation status, which were reverted upon successful antiretroviral treatment (34, 35). The reduced capacity of CD4 and CD8 T cells to upregulate Bcl-2 expression in patients with severe heart disease is in agreement with the poor STAT5 phosphorylation induced upon IL-7 stimulation found in these subjects. Although restricted to $CD8^+$ T cells, CD25 upregulation, being another STAT5 downstream function, was also impaired in subjects with chronic Chagas disease.

The alterations observed in the IL-7/IL-7R system in long-term *T. cruzi*–infected subjects could explain the diminished frequencies of *T. cruzi*–specific T cells and the increased levels of T cell apoptosis, previously found in subjects in the chronic phase of the infection (3, 4). Moreover, more than one mechanisms of immune exhaustion might be triggered during chronic *T. cruzi* infection, as indicated by our previous findings showing increased expression of CTLA-4 by IFN-γ–producing $CD4^+$ T cells responsive to *T. cruzi* Ags and in tissues from patients with severe cardiomyopathy (36).

Although most IFN-γ–responsive T cells to *T. cruzi* Ags express the IL-7R α-chain (D.P.-M., personal communication), it is possible that the function and expression of the IL-7R might be dampened over time. In a mouse model of chronic *T. cruzi* infection, a population of $CD8^+CD127^{hi}CD62L^+CD122^+$ T cells specific for *T. cruzi* was maintained following transfer into naive mice (37). This T cell population was capable of producing IFN-γ

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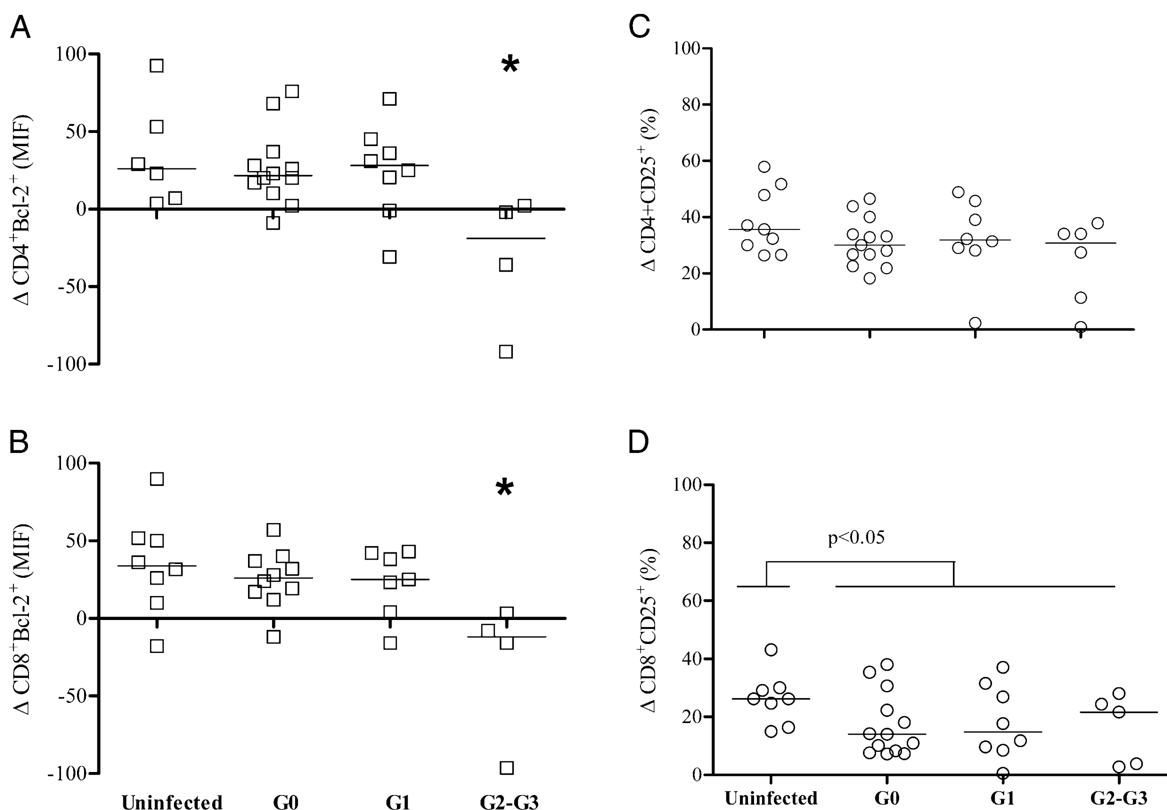

FIGURE 5. Impaired IL-7 functional responses in $CD4^+$ and $CD8^+$ T cells from chronic Chagas disease patients. PBMCs from healthy donors ($n = 9$) and chronically *T. cruzi*–infected subjects (G0, $n = 13$; G1, $n = 8$; G2 and G3, $n = 6$) were cultured for 2 d in complete medium in the presence or absence of 10 ng/ml IL-7. Each point represents the differences in Bcl-2 MFI (Δ Blc-2 MFI) (**A** and **B**) and in the percentage of $CD25^+$ T cells (Δ% CD25) (**C** and **D**) between IL-7-stimulated and unstimulated samples in $CD4^+$ or CD8 T cell compartments. (A and B) ANOVA followed by a Bonferroni test for multiple comparisons. *$p < 0.05$ for G2 and G3 compared with G0, G1, and uninfected subjects. (D) Mann–Whitney *U* test between *T. cruzi*–infected and uninfected subjects. $p < 0.05$ compared with *T. cruzi*–infected subjects.

upon restimulation with *T. cruzi* Ags and expanded in response to challenge infection, indicating that these cells are functionally responsive upon Ag re-encounter (37). However, the mouse model of chronic *T. cruzi* infection does not lead to immune exhaustion (37, 38).

In conclusion, parasite persistence in chronic Chagas disease alters the IL-7/IL-7R pathway system, which might represent another potential mechanism of T cell exhaustion in chronic *T. cruzi* infection.

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Disclosures

The authors have no financial conflicts of interest.

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